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6651 GLYPHOSATE HERBICIDE (PROPOSED)*

6651 A. Introduction

1. Sources and Significance

Glyphosate[*N*-(phosphonomethyl)glycine] is a broad-spectrum, nonselective, postemergence herbicide that has found widespread agricultural and domestic use. It is sold as a terrestrial and aquatic herbicide under the trade names Roundup® and Rodeo®. Because of low mammalian toxicity (LD50 = 1568 mg/kg rats; oral) there is less concern about water and food contamination than with other pesticides, but the nonselectivity of the herbicide can make nontarget phytotoxicity a problem. Glyphosate's (GLYPH) major metabolite is aminomethylphosphonic acid (AMPA). Contamination of water can occur through runoff and spray drift.

2. Selection of Method

Several methods for determination of GLYPH and AMPA in environmental samples have been developed; those using liquid chromatography are the most precise and accurate. GLYPH and AMPA are not good chromophores or fluorophores and their electrochemical or conductometric detection have not been demonstrated. Sensitive and selective detection has been achieved with the post column reaction/fluorometric method.[1-3] The absence of a sensitive liquid chromatography technique for confirmation necessitates the use of two different stationary phases.

The liquid chromatographic method presented in 6651B is accurate and precise[3] and includes confirmation by using two columns. Gas chromatography/mass spectrometry confirmation[4] has been used when structural confirmation is required, but the method has not been tested on residues in natural waters.

3. References

1. MOYE, H.A., C.J. MILES & S.J. SCHERER. 1983. A simplified high-performance liquid chromatographic procedure for the determination of glyphosate herbicide and (aminomethyl)phosphonic acid in fruits and vegetables employing postcolumn fluorogenic labeling. *J. Agric. Food Chem.* 31:69.
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* Approved by Standard Methods Committee, 1991.

6651 B. Liquid Chromatographic Postcolumn Fluorescence Method

1. General Discussion

a. Principle: GLYPH and AMPA are separated by anion- or cation-exchange chromatography and measured by postcolumn fluorescence derivatization. The postcolumn reactions consist of oxidation of GLYPH (a secondary amine) to glycine (a primary amine) by hypochlorite solution. Glycine then reacts with an *o*-phthalaldehyde (OPA) and mercaptoethanol (MERC) mixed reagent to form an isoindole that is measured fluorometrically. AMPA (a primary amine) reacts directly with the OPA/MERC reagent and is detected (with decreased sensitivity) in the presence of hypochlorite.

b. Interferences: No matrix interferences in water are known. GLYPH degrades in chlorinated water. GLYPH also is known to sorb strongly to minerals and glass surfaces.

c. Minimum detectable concentration: Limit of detection using this method is 25 μg/L for GLYPH and AMPA by direct injection and 0.5 μg/L with the concentration step.

2. Sampling and Storage

Collect a 500-mL representative sample in a polypropylene container. Treatment of sample to remove residual chlorine will prevent glyphosate losses during storage. Destroy chlorine by adding 100 mg/L sodium thiosulfate. Store samples at 4°C away from light and analyze within 2 weeks.

3. Apparatus

a. High-performance liquid chromatograph (HPLC): An analytical system with pump, injector, detectors, and compatible strip chart recorder. Preferably use a data system for measuring peak areas and retention times. Use system capable of injecting 200-μL portions. See Figure 6651:1.

1) *Analytical columns:* Use either a cation exchange resin[1] or an anion-exchange resin[2] packed in a 4.6-mm × 25- to 30-cm

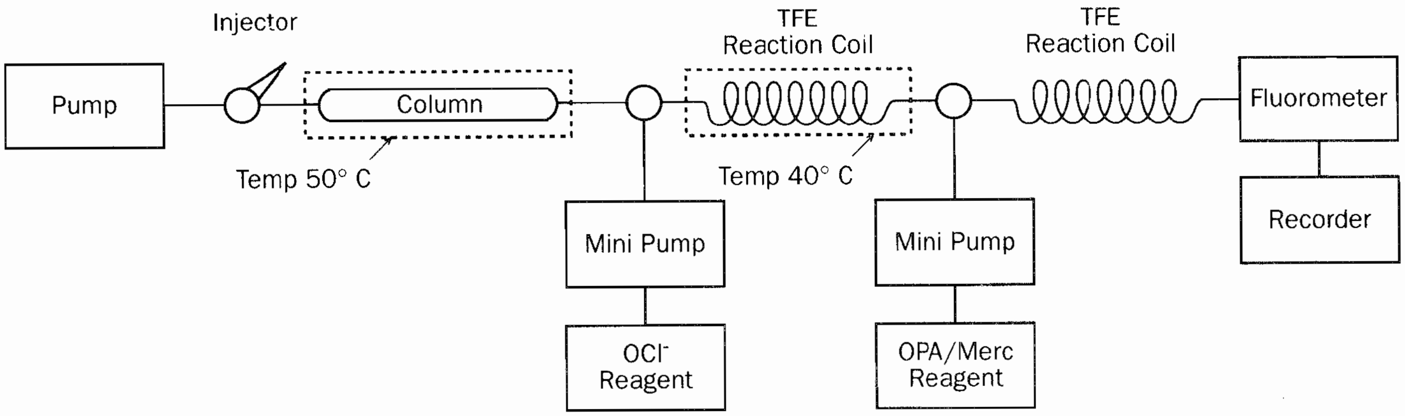

Figure 6651:1. Schematic of postcolumn reaction HPLC system.

column.* Heat columns to between 50 and 60°C to obtain maximum efficiency.

2) *Postcolumn reactor:* Use system consisting of two separate pumps capable of delivering reaction solutions at 0.1 to 0.5 mL/min and able to withstand pressures of up to 2000 kPa. Include two woven 1-mL TFE reaction coils[3] (0.5-mm ID × 1.4-mm OD × 5 m) with one maintained at 40°C. Turnkey postcolumn reactor systems are available commercially.

3) *Fluorescence detector:* Use filter or grating fluorimeter capable of sensitively and selectively measuring the isoindole derivative, with excitation wavelength 230 nm (deuterium), 340 nm (quartz halogen or xenon), and emission wavelength 420 to 455 nm.

4. Reagents

a. Reagent water: See Section 1080.4*b*.

b. Phosphoric acid, H_3PO_4, conc.

c. Sulfuric acid, H_2SO_4, conc. Prepare anion-exchange mobile phase by adding 26 mL conc H_3PO_4 and 2.7 mL conc H_2SO_4 to 5 L water.

d. Hydrochloric acid, HCl, conc.

e. Methanol, CH_3OH, tested on HPLC and verified to give no impurity peaks.

f. Potassium dihydrogen phosphate, KH_2PO_4. Prepare cation-exchange mobile phase by dissolving 0.68 g KH_2PO_4 in 1 L methanol-water (4:96). Adjust to pH 2.1 with conc H_3PO_4. Filter through a 0.22- or 0.45-m membrane filter and degas.

g. Disodium ethylenediamine tetraacetate dihydrate, EDTA sodium salt solutions: Prepare a 0.001*M* solution by dissolving 0.37 g EDTA dihydrate in 1.0 L water and filter through a 0.22- or 0.45-μm filter. Prepare a 0.03*M* solution by dissolving 11.2 g EDTA dihydrate in 1.0 L water and filtering through a 0.33- or 0.45-μm filter.

h. Sodium chloride, NaCl.

i. Sodium hydroxide, NaOH.

j. Calcium hypochlorite, $Ca(OCl)_2$, 70.9% available chlorine.

k. Oxidation reagent: Dissolve 0.5 g$Ca(OCl)_2$ in 500 mL water with rapid magnetic stirring for 45 min. In a 1.0-L volumetric flask, dissolve 1.74 gK H_2PO_4, 11.6 g NaCl, 0.4 g NaOH, and 10 mL stock $Ca(OCl)_2$ solution. Dilute to volume, mix well, and filter through a 0.22 or 0.45 μm filter.

l. o-phthalaldehyde, $C_6H_4(CHO)_2$, OPA.

m. 2-mercaptoethanol, $HSCH_2CH_2OH$, MERC.

n. Boric acid powder, H_3BO_3.

o. Potassium hydroxide, KOH.

p. Fluorogenic labeling reagent: Dissolve 100 g boric acid and 72 g KOH in about 700 mL water in a 1.0-L flask. This takes 1 to 2 h. Add 0.8 g OPA dissolved in 5 mL methanol. Add 2.0 mL MERC. Mix well.

q. Glyphosate analytical standard, N-(phosphonomethyl) glycine, 99% or greater.

r. Aminomethylphosphonic acid analytical standard, 99% or greater.

s. Glyphosate and AMPA fortification standards: Prepare a solution containing both 0.1 mg GLYPH/mL and 0.1 mg AMPA/mL in water. Make working solutions of 10.0 and 1.0 μg/mL by serial dilution of this stock solution. Store in a refrigerator, in a polypropylene bottle. Prepare fresh monthly.

t. Glyphosate and AMPA HPLC calibration standards: Prepare a solution containing both 0.1 mg GLYPH/mL and 0.1 mg AMPA/mL in 0.001*M* disodium EDTA solution. Make working solutions of 1.00, 0.50, 0.10, 0.05, and 0.025 μg/mL by serial dilution. Store in a refrigerator, in polypropylene bottle. Prepare fresh monthly.

5. Procedure

a. HPLC operation: Equilibrate column at 50°C with mobile-phase flow rate of 0.5 mL/min (see Figure 6651:1). Use an approximate flow rate of 0.5 mL/min for the oxidant and 0.3 mL/min for the OPA-MERC reagent but adjust rates to obtain maximum response. While GLYPH reaches a maximum response at some flow rate of oxidative solution, the AMPA response decreases with any addition of this reagent. Thus, an oxidative reagent flow rate that gives an equal response for both GLYPH and AMPA simultaneously is considered optimum for simultaneous measurements. Reagent flow rates will differ for different mobile phases. Establish a standard curve by injecting calibration standards.

Approximate retention times for GLYPH and AMPA are 13.5 and 10.0 min on the anion-exchange column and 21.5 and 30.0 min on the cation-exchange column.

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* Aminex, BioRad Labs, A-9 cation exchange and A-27 anion exchange resins, or equivalent.

b. Sample preparation: No concentration is needed for samples containing 25 µg/L or more. Fortify a 9.9-mL sample portion with 0.1 mL 0.10*M* EDTA, filter through a 0.22- or 0.45-µm filter, and inject 200 µL. Fortify portions of the same samples with known amounts of GLYPH and AMPA to determine recovery. Perform duplicate injections on at least 10% of the samples with measurable GLYPH and AMPA or 10% of fortified samples to determine precision.

To concentrate samples containing less than the detection limit, transfer 250 mL to a 500-mL round-bottom flask. If suspended matter is present, filter sample through coarse filter paper.† For samples used to assess recovery, make known additions. Add 5 mL conc HCl to flask and 5 mL to sample remaining in original container. Concentrate on a rotary evaporator by slowly increasing temperature from 20 to 60°C. Before the first portion is completely evaporated, add remaining sample and two 5-mL rinses of the sample bottle. Evaporate to dryness, and if necessary, remove final traces of water with a stream of dry nitrogen. Dissolve residue in 2.9 mL of mobile phase (adjust pH to 2 if necessary) and 0.10 mL 0.03*M* EDTA solution. Filter through 0.45-µm filter to a test tube and inject into the HPLC system.

6. Calculations

Determine concentration of GLYPH and AMPA by regression analysis of the standard curve. Multiply results for samples that were concentrated by the concentration factor, 166.7 (500 mL original sample/3.0 mL), to determine the original water concentration. Report results in milligrams per liter. Report percent recovery but do not correct for recovery.

7. Quality Control

See Section 6020.

8. Precision and Bias

For six single-operator analyses, the relative standard deviation of duplicate samples (with additions from 0.5 to 5000 µg/L) ranged from 12.1 to 20% with an average of 14.9% for glyphosate. The relative standard deviation for identical AMPA concentrations ranged from 6.5 to 28.8% with an average of 14.5%.[4]

For six single-operator analyses, recoveries of glyphosate (with additions from 0.5 to 5000 µg/L) ranged from 94.6 to 120% with an average of 104.0%. Recoveries of AMPA ranged from 86.0 to 100% with an average of 93.1%.[4]

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† Whatman #1 or equivalent.